In response, applicant has amended the specification of the instant application to identify each sequence to be included in the concurrently submitted "Sequence Listing" with a Sequence ID No.

Such amendment does not constitute new matter, and applicant respectfully requests the Office to proceed with further examination on the basis of such amendment.

Respectfully submitted,

Yongzhi Yang
Registration No. (see attached)

Steven J. Hultquist
Registration No. 28,021
Attorney for Applicant

**INTELLECTUAL PROPERTY/
TECHNOLOGY LAW**
P. O. Box 14329
Research Triangle Park, NC 27709
(919) 419-9350
Attorney Reference: 4176-101

APPENDIX A

Version with Markings to Show Changes Made

In the Specification:

1. On page 2, the paragraph beginning at line 12 has been changed, as follows:

ELPs, as explained more fully in the Detailed Description of the Invention hereof (Section 5) are oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly (Sequence ID No. 1), where the guest residue X is any amino acid. ELPs undergo a reversible inverse temperature transition. They are highly soluble in water below the inverse transition temperature (T_t), but undergo a sharp (2-3°C range) phase transition when the temperature is raised above their T_t, leading to desolvation and aggregation of the polypeptide.[1, 2, 3] In previous work, McPherson et al. have exploited the inverse transition to purify recombinant poly(GVGVP) polypeptides. Previous studies have also shown that protein conjugates of poly(N-isopropylacrylamide), a synthetic polymer that undergoes a similar thermally-reversible phase transition, also retain the transition behavior of the free polymer.[5, 6, 7]

2. On page 14, the paragraph beginning at line 15 has been changed, as follows:

Another preferred ELP comprises polymeric units having the sequence IPGXG (Sequence ID No. 2), where X is as defined above.

3. On page 23, the paragraph beginning at line 26 has been changed, as follows:

The objective in this example was to design a β-turn sequence with a predicted T_t above 37°C so that an FP would remain soluble under conditions used for E. coli culture, but which could be aggregated by a small increase in temperature. Previous studies by Urry and colleagues have shown that two ELP-specific variables, guest residue(s) composition[28] (i.e., identity and mole fraction of X in the VPGXG monomer) and chain length[29] of the ELP profoundly affect the transition temperature, and thereby provide design criteria to specify the T_t for a specific application. Based on these studies, a gene was synthesized encoding an ELP sequence (Sequence ID No. 3) with guest residues valine, alanine, and glycine in the ratio 5:2:3, with a predicted T_t of ~40°C in water. The synthetic gene, which encoded 10 VPGXG pentapeptide repeats (the "10-

mer"), was oligomerized up to 18 times to create a library of genes encoding ELPs with precisely-specified molecular weights (MWs) ranging from 3.9 to 70.5 kDa. To my knowledge, these are the first examples of genetically-engineered ELPs with precisely-defined chain length and amino acid sequence, which are designed to exhibit an inverse transition at a specified temperature. Thioredoxin was expressed as a N-terminal fusion with the 10-, 20-, 30-, 60-, 90-, 120-, 150-, and 180-mer ELP sequences, and tendamistat was expressed as a C-terminal fusion to thioredoxin/90-mer ELP (Figure 1b).

4. On page 32, the paragraph beginning at line 21 has been changed, as follows:

Standard molecular biology protocols were used for synthesis and oligomerization of the ELP genes (Ausubel, et al.[32]). Monomer genes for two ELP sequences were utilized in this example. The first, ELP[$V_5A_2G_3$-10] encoding ten Val-Pro-Gly-Xaa-Gly repeats where Xaa was Val, Ala, and Gly in a 5:2:3 ratio, respectively, had been synthesized previously[37]. The second monomer, ELP[V-5] (Sequence ID No. 4), encoded five Val-Pro-Gly-Val-Gly pentapeptides (i.e., Xaa was exclusively Val). The coding sequence for the ELP[V-5] monomer gene was: 5'-GTGGGTGTTCCGGGCGTAGGTGTCCCAGGTGTGGGCGTACCGGGCGTTGGTGTTCCTG GTGTCGGCGTGCCGGGC-3' (Sequence ID No. 5). The monomer genes were assembled from chemically synthesized, 5'-phosphorylated oligonucleotides (Integrated DNA Technologies, Coralville, IA), and ligated into a pUC19-based cloning vector. A detailed description of the monomer gene synthesis is presented elsewhere[38].

5. On page 33, the paragraph beginning at line 11 has been changed, as follows:

Different ELP constructs are distinguished here using the notation ELP[X_iY_j-n], where the bracketed capital letters are single letter amino acid codes and their corresponding subscripts designate the frequency of each guest residue in the repeat unit, and n describes the total length of the ELP in number of pentapeptides. The two ELP constructs central to the present example are ELP[$V_5A_2G_3$-90] (35.9 kDa) (Sequence ID No. 6) and ELP[V-20] (9.0 kDa) (Sequence ID No. 7).